Distinguishing Nitrogen Fertilization Levels in Field Corn (*Zea mays* L.) with Actively Induced Fluorescence and Passive Reflectance Measurements*

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*Laser-induced fluorescence (LIF) is an active sensing technique capable of capturing immediate and specific indications of changes in plant physiology and metabolism as they relate to the concentration and photosynthetic activity of the plant pigments. Reflectance is a passive sensing technique that can capture differences in the concentration of the primary plant pigments. Fluorescence and reflectance were compared for their ability to measure levels of plant stress that are of agronomic importance in corn (*Zea mays *L.) crops. Laboratory LIF and reflectance spectra were made on excised leaves from field grown corn. Changes in the visible region of the spectrum were compared between groups of plants fertilized with seven different levels of nitrogen (N) fertilization. A pulsed nitrogen laser emitting photons at a wavelength of 337 nm was used as a fluorescence excitation source. Differences in maximum intensity of fluorescence occurred at 440 nm, 525 nm, 685 nm, and 740 nm. Significant separations were found between levels of N fertilization at several LIF wavelength ratios. Several reflectance algorithms also produced significant separations between certain levels of N fertilization.*

INTRODUCTION

Remote sensing technology is needed to detect stress conditions due to overfertilization or underfertilization. Sensing of immediate changes in crop physiology and metabolism could lead to methods for timely correction of the problem before irreversible damage is done. Cumulative remote measurements that reliably monitor temporal deviations from normal plant development through a season could also be an important long-term management tool. Upon sensing a stressed condition due to fertilization, a farmer could immediately correct the imbalance in that area of the field. Longer term cumulative remote sensing measures could establish a pattern of a crop's physiological stress in a field. This could indicate a need for differential fertilization amendments to certain segments of the field in subsequent years. Both options express ways of using remote sensing to monitor the utilization and effectiveness of N fertilization and other agricultural inputs to their maximum efficiency. This research is a study of the utility of LIF and reflectance algorithms as methods for sensing plant condition due to N fertilization levels.

Actively induced fluorescence and passive reflectance were investigated for their potential to detect different levels of nitrogen (N) fertilization in field grown corn (*Zea mays* L.) plants. Data was collected in the 400–800 nm region of the spectrum. Recent USDA agricultural statistics indicate that there are approximately 11 million tons of nitrogen fertilizer applied per year to crops on United States soils, which costs the American farmer over 5 billion dollars annually (Office of Governmental and Public Affairs, 1988). Nutrient availability is ranked second only to rainfall as the most

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important requirement for profitable crop production. Nitrogen assimilation is the most important plant nutritional process that a farmer manages in cultivated crops (Meisinger, 1984). Leaching and run-off of nitrogen from soil are environmentally important issues affecting the quality of our water resources (Meisinger and Randall, 1991). Overfertilization can cause excess N to leach into streams, waterways, and groundwater, while underfertilization can reduce the profit / cost ratio to an unacceptable margin. It is therefore important to optimize N fertilization.

Fluorescence

Plants contain pigments which absorb photons from sunlight that are involved in photosynthesis and other photochemical processes. The leaf pigments, when exposed to photons of certain wavelengths, can emit part or all of this absorbed energy as fluorescence at longer wavelengths. Fluorescence and / or heat emission occurs if the energy received is in excess of what can be transferred and assimilated by photosynthesis and other biochemical systems of the plant. The magnitude of the fluorescence emission is inversely related to the relative efficiency of plant photosynthesis and other biochemical systems. Fluorescence can also be an indicator of the relative concentration of certain plant constituents. Measurable fluorescence emissions can be induced by lasers (light amplification by stimulated emission of radiation). Previous studies have indicated an ability to detect plant stress with laser induced fluorescence (LIF) (Buschmann et al., 1989; Chappelle et al., 1984a). In corn and soybean (*Glycine max* Merr.) differences between the fluorescence of healthy plants and plants deficient in the major plant nutrients N, P, and K and the minor plant nutrients Ca, Mg, S, Fe, and B have been detected (Chappelle et al., 1984 b; McMurtrey et al., 1983). The LIF technique uses fluorescence information from "light" energy electron transfer interactions from pigments within the plant leaf and is able to sense immediate indications of changes in the reactions that occur in response to changes in plant physiology and metabolism.

Reflectance

Current techniques for remote sensing of vegetation are accomplished primarily through the use of spectral methods that monitor the temporal development of the reflectance properties of the plant canopy. These methods have proven useful in measuring the intensity and duration of the plant canopies' accumulation and decline. These measures track the plant canopy development as the green biomass covers units of ground (soil) area during the course of a growing season. Several indices for reflectance have been developed to monitor the vegetation green-up and senescence decline. These vegetative indices have been found to have the same mathematical equivalency (Perry and Lautenschlager, 1984), which indicates that they monitor similar biological functions. Remote sensing measures involving only reflectance spectra of intact plant canopies are limited to sensing an "ill-defined" combination of plant parameters and associated physiological factors. Reflectance measures have been successful in tracking factors that are most closely related to the plant pigment concentration of chlorophyll. A recent ratio analysis reflectance spectra technique (RARS) has shown good correlation with three primary plant pigments, chlorophyll *a*, chlorophyll *b*, and total carotenoids (Chappelle et al., 1992). In general, reflectance measurements indicate the long-term effects of stress in crop canopies that result in variations of chlorophyll concentration and percent cover of green leaf biomass; however, for the most part, reflectance cannot distinguish between different stresses or immediate metabolic changes in the plant canopies.

Objectives

The following study characterized measurements from active LIF sensing and passive reflectance sensing of leaves from a field corn canopy at various levels of N fertilization. LIF measurements theoretically should be able to detect immediate changes that are closely related to plant photosynthesis through the electron transfer kinetics of the plant pigments involved in the plants' metabolic processes. Reflectance information, on the other hand, is solely associated with the concentration of the plant pigments. Effective intact canopy sensing may require algorithms derived from several forms of remote sensing (i.e., visible and near-IR reflectance, thermal IR emissions, LIF emissions, and microwave emissions) conducted simultaneously at the same resolution, and all accumulated temporally over a growing season. Combined sensor techniques may prove useful for estimating field crop production and crop condition by hand-held sampling measurements, tractor-mounted tool bar sensors, or by large-area field measurements from aircraft or other aerial platforms. In this study, we compare and couple the effects of sensing with fluorescence and reflectance.

MATERIALS AND METHODS

Conditions of Plant Growth

Nitrogen deficiency was induced in corn plants grown in field plots at Beltsville, Maryland on a Woodstown sandy loam soil. The field was supplied with the optimal rates of the essential nutrients P at 14 kg / ha and K at 27 kg / ha. These rates are recommended by the local farm extension service and are known to produce a healthy and profitable corn crop in this production area. Dolomitic lime had been applied to the field to maintain a pH of 6.0 and supply the essential minerals of Ca and

Mg. Other essential nutrients (such as S, Fe, B, Mn, Cu, and Zn) were supplied by the natural mineralization in the parent soil. The essential nutrient N was applied at an optimal rate, at five progressively lower levels, and at one higher level as described in Table 1. Nitrogen was supplied in the form of urea [$CO(NH_2)_2$] by side-dressing the area 2 weeks after emergence at rates of 0 kg/ha, 22.5 kg/ha, 45 kg/ha, 90 kg/ha, 135 kg/ha, 180 kg/ha, and 270 kg/ha. Four replicate areas for each N level were treated in a randomized complete block design. The 180 kg/ha N rate is considered optimal for satisfying the N nutritional needs of corn in Maryland.

The corn was planted in 76 cm rows by conventional planting techniques using moldboard plow tillage. The corn variety was Hytest 318, which was planted at a density of 55,000 plants per hectare. Fluorescence and reflectance spectra were taken on leaves from the field grown corn canopy. The spectra were taken on the upper most fully expanded leaf after 7 weeks of plant growth. The corn plants were about 2 m tall at the 12-leaf pretasseling stage (Hanway, 1963). Early in the growing season, it was evident that the plot to plot variability of the four replications of the seven treatments across the field was high due to water drainage or other factors related to the soil's variability. There was less variability within individual treatment effects in the first seven-plot replication block of the experiment. Subsequent measurements were made on seven individual plants (which were classified as the seven replicates used for this study) from each of the seven N treatment plots from the first block of the field experiment.

Measurement of Photosynthesis

Rates of photosynthesis were measured in the field on the intact uppermost fully expanded leaf on the corn plant. The rates of CO_2 exchange were determined with a LICOR Model 6200 infrared gas analyzer operated in the closed mode. The ambient CO_2 concentration was 330 ppm. The temperature within the photosynthesis chamber did not exceed 27°C, while the relative humidity remained at approximately 70%. There was no water stress evident in the field at the time the measurements were taken. The CO_2 uptake measurements were made on a leaf area of 2.54 cm^2 from seven plants from each treatment.

Table 1. Field Corn N Treatments

Percent of Optimal Recommended N Rate (%)	*N Applied (kg/ha)*
150.0	270.0
100.0	180.0
75.0	135.0
50.0	90.0
25.0	45.0
12.5	22.5
0.0	0.0

Configuration of the LIF Equipment

The primary excitation source for the LIF was a Laser Science pulsed nitrogen laser. This laser, which emits at 337 nm, was operated at 20 Hz with a power output of 250 J per pulse and a pulse width of 3 ns. The laboratory configuration was housed in a mobile van and consisted of three main elements: a fluorescence excitation source, a signal receiver, and a data acquisition system. The laser beam was used as the excitation source and was focused on one bundle of a bifurcated fiber optics. Adaxial sides of detached plant leaves were placed into a leaf holding apparatus, which held the leaf 1 mm away and perpendicular to the end of a 5 mm diameter bifurcated fiber optics. One bundle carried the excitation signal to the leaf target. The other bundle detected the fluorescence signal by returning it to the entrance slit of a scanning monochromator. The fluorescence intensity as a function of wavelength was detected with a sensitive gallium arsenide photomultiplier tube. Reflected light from the laser was prevented from entering the detection side by attaching a 390 nm cutoff filter in front of the monochromator slit. A gated boxcar integrator system was used to trigger the laser pulse and capture the 10 ns pulsed signal at a reproducible point on the peak of each excitation pulse. Fluorescence was measured as relative fluorescence intensity (RFI) and the nonlinearity in response of the system was calibrated to the output of a standard tungsten lamp. The signal was fed into a computer via an A/D converter for generating real time spectra. Stanford Research Systems (SR265) data acquisition software was used to process the data through the computer interface with the A to D board for storing data for subsequent analysis. The uppermost fully expanded leaf from seven plants in each treatment that had been measured for photosynthesis was cut from the plant, taken immediately to the laboratory van, and measured for fluorescence.

Measurement of Reflectance

Reflectance scans were acquired with a double beam Perkin Elmer spectrophotometer in the diffuse reflectance mode, set at a resolution of 5 nm, and scanned from 400 nm to 800 nm at a speed of 120 nm/min. The adaxial surface of the seven replications of freshly excised leaves from the seven N treatments were attached to the 2 mm × 3 mm sample port which gathers diffuse reflectance. A barium sulfate panel was used in the reference port.

Measurement of Plant Pigments

Leaf disk from the N treatments leaf samples were cut using a number 12 cork bore. Chlorophyll and carot-

enoid extractions were run and calculated on these 2.54 cm^2 leaf disks using a method similar to the one described by Lichtenthaler (1987) except that dimethyl sulfoxide (DMSO) was used as an extraction solvent. This extraction method yields most of the nonstructural components in the plant.

Measurement of Corn Yield

Grain from 10 plants in each treatment were used to calculate the yield per treatment when the corn crop was mature and ready for harvest. A different set of plants were used to calculate the yield of the treatments than were used for the other plant measurements. The yield measurements could not therefore be used for correlation analysis with the other measurements. Yield in kg / ha was established by multiplying grain weight per plant by plant population counts per hectare (Table 3).

RESULTS AND DISCUSSION

Photosynthesis

Photosynthetic rates for the optimal (100%) rate of N fertilization, 150% of the optimal N fertilization, and 75% of the optimal N fertilization were not significantly different from each other at the 5% level of significance by Student–Newman–Keuls (SNK) multiple range testing (Table 2). N fertilization at 50% of the optimal N fertilization and below were significantly different from the higher levels of N fertilization. Several of the lower levels of N fertilization were not significantly different from one another and overlapped in multiple range testing of the means.

Pigment Concentration

Multiple range test of the means for extracted chlorophyll *a*, chlorophyll *b*, total carotenoids, chlorophyll *a* + *b*, and total primary pigment concentrations followed the same general pattern as the photosynthesis data except that there appeared to be more sensitivity in separating the lower levels of N fertilization treatments with the extracted pigment data (Table 2). Chlorophyll *b* mean concentrations also gave significant separation at higher levels of N fertilization than did chlorophyll *a* or the other pigment concentration combinations.

Table 3. Field Corn Yield

Treatment of Optimal Rate (%)	*1991 (kg / ha)*
150.0	10484 a
100.0	10853 a
75.0	10363 a
50.0	8559 b
25.0	6452 c
12.5	5935 c
0.0	4440 d

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

Field Corn Yield

The three highest levels of N fertilization were not statistically different from one another in grain yields. In general, mean grain yield followed the same statistical trend as did the mean rates of photosynthesis and the mean concentrations of the primary plant pigments (Table 3). Direct statistical comparison by correlation between the grain yields, the other plant parameters, and the spectral data could not be made because a different set of plant samples were used for the N treatment levels grain yield measurements.

Fluorescence

Relative fluorescence intensity of field grown corn revealed spectra separations between plant spectra grown at different N fertilization levels (Fig. 1). At 440 nm, 525 nm, 685 nm, and 740 nm wavelengths, Chappelle et al. (1984a) have reported predominate bands in the spectra where the steady state fluorescence intensity appears to be most sensitive to changes in plant condition. These same areas also appeared to be the most sensitive in measuring differences in corn plant condition due to the application of different levels N fertilization in the field. However, the only single wave-band area centered on the peak at 685 nm showed statistically significant differences between the zero level of N application and the two highest levels of N application (Table

Table 2. Means of Photosynthesis and Extracted Plant Pigment Concentrations from Field Corn

Optimal Fert. Rate (%)	*Photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)*	*Cha (µg / mL)*	*Chb (µg / mL)*	*Cha + b (µg / mL)*	*Total Carotenoids (µg / mL)*	*Total Pigments (µg / mL)*
150.0	116.12 a	30.94 a	12.04 a	42.98 a	3.80 a	46.78 a
100.0	137.02 a	30.19 a	11.22 a	41.41 a	4.00 a	45.41 a
75.0	116.24 a	27.39 a	9.05 b	36.44 a	4.32 a	40.76 a
50.0	74.92 b	21.53 b	6.26 c	27.79 b	4.27 a	32.06 b
25.0	52.96 bc	18.68 c	5.16 c	23.84 b	4.13 a	27.97 b
12.5	45.78 bc	17.81 c	4.78 c	22.59 b	4.25 a	26.84 b
0.0	19.84 c	11.04 d	2.85 d	13.89 c	2.89 b	16.78 c

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

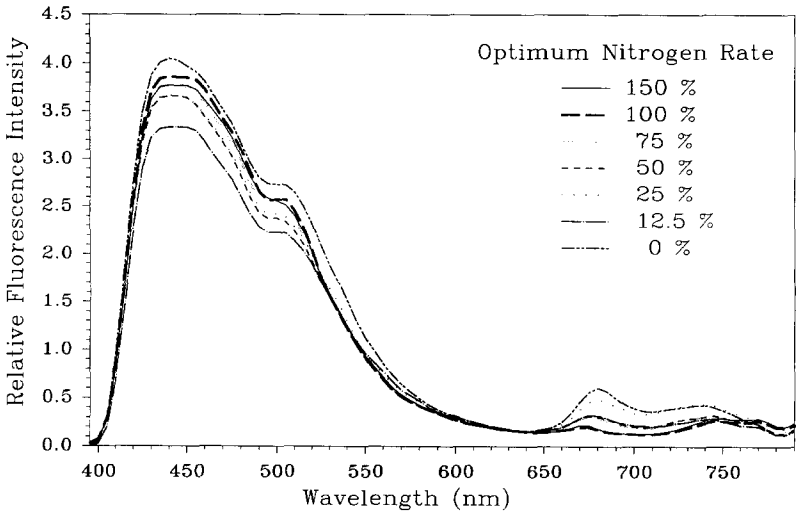

Figure 1. Mean relative fluorescence intensity spectra from 400 nm to 800 nm of field grown corn treatments subjected to different levels of nitrogen fertilization.

4). Detection of significant differences between fertilization levels of N on field corn was realized with several two-band ratio combinations (Table 5). The ratio of the relative fluorescence intensity (RIF) at 440 nm over 685 nm was able to separate the lowest four fertilizer rates from the highest two fertilizer rates at the 5% level of significance. Seventy-five percent of the optimal N rate could be statistically separated from the optimal N (100%) rate, but 150% of the optimal N rate was not statistically different from the optimal N (100%) rate. The fluorescence ratio of 525/685 nm and the ratio of 740/685 nm both were able to distinguish significant differences between the two highest levels of N fertilization and the five lowest levels of N fertilization. Lichtenthaler and Rinderle (1988), Lichtenthaler et al. (1990), and Rinderle et al. (1991) have noted a similar response to the inverse of our ratio of 740/685 nm. The 525/685 nm and 740/685 nm ratios had the same trend of mean fluorescence for the top three N levels as did the extracted chlorophyll b mean values (Table 5). These fluorescence ratios appear to be able to detect the type of metabolic differences that are associated with the significant decline in chlorophyll *b* that occur at just below the optimal N (100%) rate. It is encouraging that at 740 nm there was a trend in the mean fluorescence intensities at upper levels of N fertilization whereby

Table 4. Means of Optimal Fluorescence Bands from Field Corn

Optimal Fert. Rate (%)	*Relative Fluorescence Intensity*				
	F440	*F525*	*F600*	*F685*	*F740*
150.0	3.77	1.78	0.29	0.18 b	0.29
100.0	3.85	1.77	0.27	0.18 b	0.16
75.0	3.76	1.75	0.30	0.30 ab	0.32
50.0	3.65	1.73	0.27	0.31 ab	0.32
25.0	3.65	1.80	0.30	0.49 ab	0.43
12.5	3.34	1.72	0.30	0.32 ab	0.28
0.0	3.95	2.13	0.33	0.64 a	0.43
	N.S.	N.S.	N.S.		N.S.

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

Table 5. Means of Optimal Fluorescence Band Ratios from Field Corn

Optimal Fert. Rate (%)	*Relative Fluorescence Intensity*			
	F440/F685	*F440/F740*	*F525/F685*	*F740/F685*
150.0	22.34 ab	14.56 ab	10.29 a	1.59 a
100.0	25.22 a	15.29 a	11.14 a	1.64 a
75.0	16.22 bc	13.67 ab	7.37 b	1.17 b
50.0	13.01 c	11.88 ab	6.16 b	1.09 b
25.0	10.29 c	9.97 b	5.14 b	1.00 b
12.5	13.54 c	12.46 ab	6.63 b	1.05 b
0.0	8.71 c	10.32 ab	4.44 b	.79 b

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

the optimal N fertilization rate produced the lowest fluorescence intensity, lower even than the corn fertilized at 150% of the optimal N fertilization rate. Conversely, the mean fluorescence intensity trend at 440 nm was higher for the optimal N fertilization rate than the mean fluorescence at 440 nm for 150% of the optimal N fertilization rate. This trend was also repeated in all of the fluorescence ratio mean values, which would indicate that with enough replication there may be potential for detecting over fertilization (Table 5). Temporal collection of this data over the growing season may greatly improve separability of these fertilization levels.

The effects on fluorescence at 685 nm and 740 nm are known to equate to the activity and electron transfer of chlorophyll in photosystem II and in photosystem I, respectively (Kok, 1976). Fluctuations also occur in fluorescence at 440 nm and 525 nm in response to stress. These fluctuations are probably due either to a buildup or shortage of compounds (such as NADPH, vitamin K, riboflavin, and β-carotene) that are known to absorb in this region of the spectrum, or to change oxidation states in response to photon absorption under different levels of stress, resulting in a differential fluorescence reaction in the 440–525 nm region of the spectrum (Chappelle et al., 1991). Changes in fluorescence can occur in response to metabolic and physiological imbalances in the plant metabolism. Undoubtedly, many of these imbalances ultimately have an effect on the metabolic and photosynthetic efficiency and can result in an immediate fluorescence response in live plants. McMurtrey et al. (1993) have reported evidence of a strong fluorescence signal in the 440–525 nm region of dry crop residue, where electron transfer activity does not come from the wet chemistry of biochemical interactions. A large portion of this signal may be due to structural components that could also vary under stress conditions in living plants.

There were two primary effects which influence RFI values at 685 nm and 740 nm; 1) the effect of chlorophyll concentration on fluorescence intensity and 2) the effect of photosynthetic efficiency on fluorescence intensity. Both of these effects are evident in the changes in the fluorescence intensity from withholding N from corn plants. In general, a decrease in chlorophyll concentration can cause a decrease in fluorescence intensity at both 685 and 740 nm (McMurtrey et al., unpublished data). Decreases in metabolic efficiency that upset the electron transfer balance can result in an immediate increase in fluorescence at 685 nm and to a lesser degree at 740 nm. The concentration effect and metabolic effect have an inverse relationship. At times each may have a counteracting effect on the resulting fluorescence intensity at the 685 nm and 740 nm wavelengths. If the pigment concentrations are different and are the predominant factor influencing fluorescence at the time of the measurement, then we can expect that fluorescence at 685 nm will decrease with decreasing chlorophyll concentration. If decreased metabolic and photosynthetic efficiency at the time of the measurement override the influence of chlorophyll concentration, then we can expect fluorescence intensity at 685 nm to increase with declining rates of N application. Therefore, the *in situ* fluorescence response of live plants in this region is the net result of the plants metabolic electron transfer efficiency, which results from the leaf chlorophyll concentration and the concentration and electron transfer balance of the plants other biochemical and photochemical activities. This metabolic electron transfer efficiency can be monitored through fluorescence.

Reflectance

Mean percent reflectance of field grown corn leaves also revealed several areas of spectral separation between plants grown at different N fertilization levels (Fig. 2). Maximum chlorophyll absorption is known to

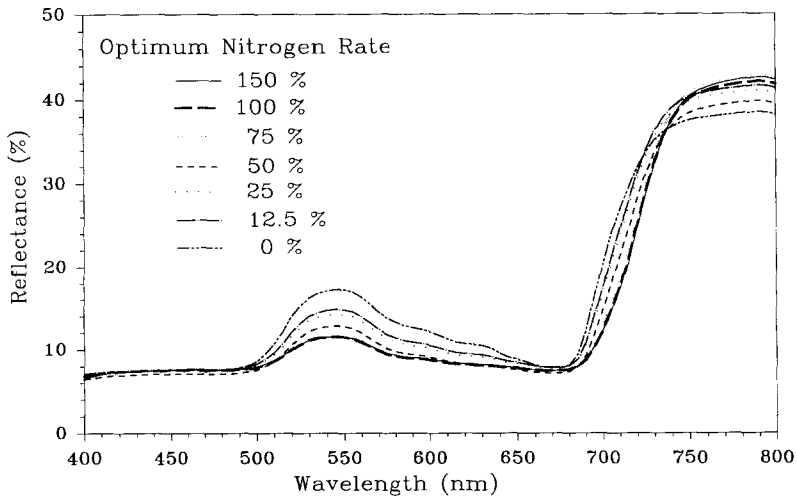

Figure 2. Mean percent reflectance intensity spectra from 400 nm to 800 nm of field grown corn subjected to different levels of nitrogen fertilization.

Table 6. Means of Optimal Reflectance Bands from Field Corn

Optimal Fert. Rate (%)	Percent Reflectance					
	R500	R550	R650	R675	R700	R760
150.0	0.08	0.12 d	0.08 ab	0.08	0.12 d	0.42
100.0	0.08	0.12 d	0.08 ab	0.08	0.12 d	0.41
75.0	0.08	0.12 d	0.08 b	0.07	0.13 d	0.42
50.0	0.08	0.13 cd	0.08 b	0.07	0.15 cd	0.39
25.0	0.08	0.14 bc	0.08 ab	0.08	0.17 bc	0.40
12.5	0.08	0.15 b	0.08 ab	0.08	0.18 b	0.41
0.0	0.09	0.17 a	0.09 a	0.08	0.20 a	0.38

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

occur in the 630–690 nm region of the spectrum. Considering nitrogen's role in the formation of the chlorophyll molecule, any differences in reflectance in this area may be attributed to the N fertilization treatment effects. Significant differences between treatments in the 550 nm band areas of the reflectance spectrum could be explained by the maximum reflectance and minimum absorption of chlorophyll (Table 6). The differences between treatments in the 700 nm region can be explained as being due to the chlorophyll concentration effects on the "red-edge shift" (Chappelle et al., 1991). The 550 nm and 700 nm wavelengths had the strongest single wavelength associations with the chlorophyll *a* concentration ($r^2 = 0.68$). Associations between single band reflectance and photosynthesis were as high as $r^2 = 0.52$ (Table 9).

Several ratio analysis reflectance spectral (RARS) algorithms have been shown to have a good association with the primary plant pigments of chlorophyll *a*, chlorophyll *b*, and total carotenoid concentration (Chappelle et al., 1992). Our study also gave statistically significant separations of the RARS derived primary pigment concentrations between corn treatments fertilized with different levels of N (Table 7).

Combined Algorithms

Algorithms using a combination of fluorescence and reflectance did a creditable job of separating groups of means of N treatment levels (Table 8). These algorithms did not separate the treatment groups as well as the extractable chlorophyll *a* and chlorophyll *b* mean values did. Fluorescence and combination algorithms had moderately significant associations with chlorophyll concentration ($r^2 = 0.42$ and below) and weaker associations ($r^2 = 0.34$ and below) with photosynthesis (Table 9).

Table 7. Measurements from Field Corn

Optimal Fert. Rate (%)	Relative Concentration Algorithm			
	RARS Cha	RARS Chb	RARS Carotenoids	NDVI
150.0	36.84 a	7.88 a	3.62 a	0.69 ab
100.0	36.90 a	7.92 a	3.59 a	0.69 ab
75.0	34.68 a	7.61 a	3.58 a	0.70 a
50.0	29.58 b	6.53 b	3.08 b	0.69 ab
25.0	28.50 b	5.84 bc	2.89 b	0.68 ab
12.5	27.48 b	5.45 c	2.78 b	0.67 ab
0.0	23.28 c	4.43 d	2.22 c	0.66 b

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

CONCLUSION

Information from plants spectral attributes in LIF spectra can be used to monitor different functional dimensions of plant physiology than the information from the reflectance forms of nondestructive sensing of plants. The areas of the spectrum from 400 nm to 800 nm where there is activity from LIF spectra of plants are different from the areas of activity in reflectance spectra of plants. Each method contains similar information about the concentration of the primary plant pigments chlorophyll *a* and chlorophyll *b* and different information about the physiological properties and the metabolic status of plants. LIF algorithms give an acute measure of the electron transfer capacity of plant pigments at 440 nm, 525 nm, 685 nm, and 740 nm as they relate to the current *in situ* metabolic status and biochemistry of the plant. Structural components could cause part of the variability in the 440–525 nm LIF region in plants with different levels of stress. Diffuse plant reflectance, on the other hand, is the result of changes in the primary plant pigment concentrations and the resulting changes in the plants architecture as plant metabolism changes. These reflectance effects are only evident after a period of chronic stress in the plants environment. Significant differences due to different levels of N fertilization in field grown corn plants indicate that distinguishing plants with gross N deficiency from well-fertilized plants is possible with both LIF and reflectance algorithms. Detection of adjacent levels of nutrient stress by LIF show promise in the establishment of an ordered trend in the ranking of the fertilizer treatments, starting with the optimal N rate and declining to progressively lower rates. It is encouraging that the overfertilization by 50% produced a fluorescence intensity trend that was lower than the optimal N rate

Table 8. Means of Combined Fluorescence–Reflectance Band Ratios from Field Corn

Optimal Fert. Rate (%)	*Relative Intensity Algorithm*	
	F740 / (F685 / RARSA)	*F440 / (F685 / RARSA)*
150.0	1.01 a	13.77 a
100.0	0.97 a	15.54 a
75.0	0.68 b	9.40 b
50.0	0.54 bc	6.48 bc
25.0	0.49 bc	6.44 bc
12.5	0.48 bc	4.92 bc
0.0	0.31 c	3.45 c

Within-column means followed by the same letter are not significantly different by SNK multiple range test P (0.05).

with the fluorescence band ratios. In the upper levels of N fertilization, where chlorophyll concentration and photosynthetic rate were not significantly different, the fluorescence ratios of bands 740 / 685 nm or 525 / 685 nm were able to detect significant early indications of metabolic stress in the 75% rate of N fertilization versus the nonstressed 100% rate of N fertilization. This could be considered to be a threshold level of metabolic and photosynthetic inefficiency caused by N deficiency stress as measured by fluorescence, whereby, the concentration of the primary plant pigment chlorophyll *a* has not as yet changed, but where the kinetics of the electron transfer through the plants other biochemical cycles have just become impaired. The fluorescence intensity presumably increased as a result of a change or imbalance of the oxidation states of the electron acceptors.

Overfertilization of N by 50% of the optimal rate (the 150% N rate) caused ranked trends in the remotely sensed spectral intensities that were below the optimal N (100%) rate for the majority of the fluorescence and reflectance algorithms. This same trend was also evident in the photosynthetic rate. From a practical agronomic standpoint, farm managers need to be able to distinguish the four highest levels of nitrogen fertilization, since farmers do not usually apply less than 50% of the recommended rate in most areas of crop production. This was successfully achieved in this study in part by

Table 9. Correction Analysis for Physical vs. Spectral Corn Attributes (r^2)[a]

	Chlorophyll a	*Chlorophyll b*	*Carotenoids*	*Photosynthesis*
*R*420	ns	ns	ns	ns
*R*500	ns	ns	ns	ns
*R*550	(−)0.67**	(−)0.58**	(−)0.12*	(−)0.45**
*R*650	(−)0.55**	(−)0.41**	(−)0.14**	(−)0.37**
*R*675	ns	ns	ns	ns
*R*700	(−)0.69**	(−)0.59**	ns	(−)0.52**
*R*760	0.15*	ns	ns	0.18**
RARS Cha	0.77**	0.76**	ns	0.55**
RARS Chb	0.76**	0.69**	ns	0.55**
RARS Cart.	0.77**	0.66**	0.12*	0.61**
*F*440	ns	ns	ns	ns
*F*525	ns	ns	ns	ns
*F*685	(−)0.24**	(−)0.18**	ns	(−)0.19**
*F*740	(−)0.08*	ns	ns	ns
RAT440 / 685	(−)0.37**	(−)0.36**	ns	(−)0.21**
RAT440 / 740	(−)0.26*	(−)0.22*	ns	0.15**
RAT525 / 685	0.36**	0.35**	ns	0.24**
RAT525 / 740	0.10*	0.10*	ns	ns
RAT740 / 685	0.35**	0.34**	ns	0.23**
RAT440 / 685 *(RARS Cha)	0.45**	0.46**	ns	0.26**
RAT740 / 685 *(RARS Cha)	0.50**	0.50**	ns	0.34**

[a] *R* = reflectance wavelength, RARS = ratio analysis reflectance spectral algorithm, *F* = fluorescence wavelength, and RAT = ratio of wavelengths. A negative sign in parentheses indicates an inverse association.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

the separation of the optimal rate from 75% of the optimal rate for N fertilization with either the 525/685 nm or the 740/685 nm fluorescence ratios. The 440/685 nm fluorescence ratio was able to separate the 75% rate of N fertilization from the 50% rate of N fertilization. Several reflectance (RARS) algorithms that have been shown good relationships to the concentrations of the primary plant pigments also were able to separate the three highest levels of fertilization from fertilization at 50% of the optimal rate. Combinations of relevant fluorescence and reflectance parameters may enhance models for detection of plant stress by using both spectral reflectance and emission. Temporal profiles of remotely sensed fluorescence and reflectance from plant canopies may improve the separability of fertilization levels.

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